

Review Paper/Derleme Makale

Characterization and encapsulation methods of lime (*Citrus aurantifolia*) oil: A review

Misket limonu (*Citrus aurantifolia*) yağının karakterizasyonu ve enkapsülasyon yöntemleri, derleme

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Abstract

Objective: Lemons and limes, characterized by their heightened acidity compared to other citrus fruits, play essential roles as flavoring agents and sweeteners in various markets. Extracting valuable essential oils from limes involves both traditional and non-traditional methods. Lemons and limes, among the most intricate citrus fruit varieties to analyze, are subjects of limited scholarly discourse, with scant literature available. This article brings together the results of studies that looked at encapsulation techniques that were meant to improve the quality of components made by extracting lime species oils and how well they kept their essential oil compositions. These encapsulation techniques have the potential to be influential in realizing future food safety objectives. Their efficacy lies in their ability to safeguard the intricate characteristics of essential oils, thereby contributing to the overarching goals of ensuring product integrity and safety within the realm of food production.

Conclusion: These encapsulation techniques have the potential to be influential in realizing future food safety objectives. Their efficacy lies in their ability to safeguard the intricate characteristics of essential oils, thereby contributing to the overarching goals of ensuring product integrity and safety within the realm of food production.

Keywords: *Citrus aurantifolia* oil, bioactive components, encapsulation, extraction, methods

Öz

Amaç: Misket limonları ve limonlar diğer turuncgillere kıyasla yüksek asitlikte olup çeşitli pazarlarda tatlandırıcı ve aroma verici olarak önemli bir rol oynamaktadır. Misket limonlarından değerli uçucu yağların elde edilmesi hem geleneksel hem de geleneksel olmayan yöntemler ile sağlanmaktadır. Analiz edilmesi en zor narenciye çeşitleri arasında yer alan limon ve misket limonu, sınırlı literatürle sınırlı bilimsel araştırma konusudur. Bu makale, misket limonu türlerinin yağlarının çıkarılmasıyla elde edilen bileşenlerin kalitesini ve uçucu yağ bileşenlerini ne kadar iyi koruyup iyileştirmeyi amaçlayan kapsülleme tekniklerini inceleyen çalışmaların sonuçlarını bir araya getirmektedir.

Sonuç: Bu kapsülleme teknikleri, gelecekteki gıda güvenliği hedeflerinin gerçekleştirilmesinde etkili olma potansiyeline sahiptir. Etkinlikleri, uçucu yağların karmaşık özelliklerini koruma özelliklerine bağlıdır ve böylece gıda üretimi alanında ürün bütünlüğü ve güvenliğini sağlamaya yönelik genel hedeflere katkıda bulunmaktadır.

Anahtar kelimeler: *Citrus aurantifolia* yağı, biyoaktif bileşenler, enkapsülasyon, ekstraksiyon, yöntemler

1. Introduction

The lime (*C. aurantifolia*), said to have originated in Malaysia or the East Indian archipelago, is a hybrid created by combining *C. maxima* with *C. medica*. *C. medica* was introduced to the eastern Mediterranean in the fifth and fourth centuries BC. *C. aurantium*, *C. aurantifolia* and *C. maxima* did not reach the Mediterranean until the 10th century AD, after the Islamic conquest. The diffusion of these *citrus* varieties to the Western world began in the 10th century AD, facilitated by Muslim influences, likely via routes passing through Sicily and the Iberian Peninsula (Langgut, 2017).

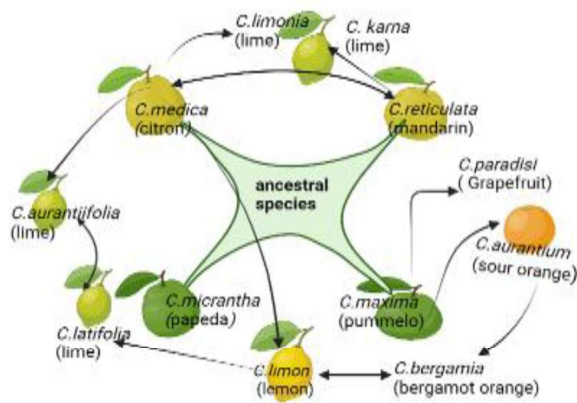


Figure 1. Pedigree of cultivable *Citrus* species

Additionally, the commercially significant *citrus* fruits within the Rutaceae family, experiencing heightened demand, trace their origin primarily to sexual hybridization involving four ancestral taxa. The phenotypic diversity observed within these familial groups predominantly arises from somatic mutations stabilized by apomixis in *citrus* species or grafting practices facilitating clonal propagation. However, utilizing the genealogical tree alone proves inadequate for comprehensively studying *citrus* domestication. Debates persist in the *citrus* taxonomy and lineage formation, particularly concerning the compatibility of genera within the *citrus* family and their related counterparts. This ongoing discourse necessitates a continual reevaluation of *citrus* taxonomy. Concurrently, extensive genomic studies are underway to elucidate the intricate phylogenetic relationships inherent in *citrus*. The broader endeavor comprehensively explores *citrus* taxonomy, diversity, origin, and domestication. Such an encompassing perspective is poised to contribute significantly to unraveling the nuanced history of *citrus* domestication (Kalita et al., 2021).

However, the European Food Safety Authority (EFSA)'s review, based on reported sound agricultural practices, believes that long-term consumption of residues arising from using potassium phosphonates is unlikely to endanger consumers' health. This is based on the residue definition of fosetyl-Al, which is made up of phosetyl, phosphonic acid, and their salts. Analytical methods, specifically tandem mass spectrometry and high-performance liquid chromatography, are recommended for detecting potassium phosphonate residues in plant matrices, including those with a high acid content, such as citrus fruits. High-fat diets contain more than 0.5 mg/kg of phosphonic acid. The EFSA also conducted a preliminary risk assessment using the updated acceptable dietary intake for phosphonic acid of 1 mg/kg body weight per day, as proposed in the EFSA decision on fosetyl. However, it is vital to note that this value has yet to get formal attention (Bellisai et al., 2021).

A study found that a combination of fish-fed *B. licheniformis* and 3% lemon peel produced extremely good growth performance in fish. This was partly due to the lemon peel's antibacterial capabilities, which successfully reduced harmful microbes in the gut flora, promoting *B. licheniformis* proliferation. The study also found that fish treated with *B. licheniformis* and/or lemon peel had significantly higher levels of blood albumin and total protein than the control group. Furthermore, including *B. licheniformis* and/or lemon peel in the diet improved humoral and cutaneous mucus immunity and antioxidative responses to *Aeromonas hydrophila* infection in fish (Sadeghi et al., 2021). Supplementation of probiotics and lemon peel reduced serum malondialdehyde levels, indicative of decreased oxidative stress and increased activity of antioxidant enzymes. This collective evidence underscores the potential synergistic benefits of incorporating probiotics and lemon peel in fish diets for growth enhancement and improving immune responses and antioxidant capabilities (Sadeghi et al., 2021).

Encapsulation technology is currently used in many different sectors, such as pharmacology, chemistry, cosmetics, food, and paints. Furthermore, this technology is becoming increasingly crucial in the world and can be a

means for future research (Calderón-Oliver and Ponce-Alquicira, 2022). This review summarizes recent research on extraction (Table 1) and

encapsulation methods for recovering bioactive compounds from *C. aurantifolia* species, commonly known as lime.

Table 1. Summary of extraction studies with lime

Extraction methods	Solvent	Devices	Components	Percentage(oils)	References
Cold Pressing	-	GC-FID*	γ -Terpinene	13.09	(Liu et al., 2022)
			Limonene	54.63	
			β -Pinene	10.21	
Cold Pressing	Ethanol <i>n</i> -hexane	GC/FID* GC/MS* UV/Vis*	Limonene	50.6	(Bitterling et al., 2022)
			γ -Terpinene	8.96	
			<i>p</i> -Cymene	5.8	
Hydro-distillation	Water	GC-MS*	<i>d</i> -Limonene	35.98	(Sandra et al., 2020)
			β -Pinene	9.02	
			Terpineol.	8.12	
Supercritical carbon dioxide	Gas	GC/MS* UV/Vis*	Limonene	59.10	(Akolade et al., 2020)
			β -Pinene	18,07	
			γ -Terpinene	10,57	
Steam distillation	Acetic acid chloroform pectin	GC/MS*	Limonene	48.43	(Etta-Francis et al., 2022)
Ultrasonically assisted extraction	Methanol	CapLC*	Limonene Linalool Farnesene α -Pinene Myrcene	persample, different concentrations	(Ponce-Rodriguez et al., 2021)
Hydro-distillation	<i>n</i> -Hexane	GC-MS*	δ -Limonene Citral β -Pinene Cosmene	22.22 14.60 14.21	(Pratiwi et al., 2022)
Hydro-distillation	<i>n</i> -Hexane	GC-MS*	<i>d</i> -Limonene Cyclohexene 4-Methylene-1-(1-methylethyl)	30.34 24.71 8.89	
			Citral β -Citral	6.38	
Steam distillation	Water	GC-MS*	α -Terpinol Limonene 3-Terpinen-1-ol β -Terpineol	44.74 11.88 4.5 3.18	(de Paiva Silva et al., 2023)
Hydro-distillation	Water	GC-MS*	Limonene	44,69 \pm 2,11	
			γ -Terpinene	16.22 \pm 1.05	

*Gas chromatography-flame ionization detector (GC-FID), mass spectrometry (GC/MS), spectrophotometric analysis (UV/VIS), capillary liquid chromatography (CAPLC), high-performance liquid chromatography (HPLC)

2. Bioactive components of lime oil

2.1. Antioxidants

The isolation of antioxidants from Mediterranean plants is an appropriate alternative (Pavlić et al., 2021). Besides, plant extracts were found to be an important source of chemical compounds having powerful antioxidant activity (Rojas and Buitrago, 2019).

C. aurantifolia Swingle is recognized as the inaugural variety of lime designed to withstand cold conditions, while *C. latifolia* is acknowledged as the authentic lime. Varieties cultivated in tropical regions exhibit larger physical dimensions

compared to their counterparts. People have historically attributed the fruit's efficacy in preventing scurvy to its consumption. Notably, lime peels and pulp, characterized by their thinness, undergo drying processes in lemon processing plants. These by-products are premium sources for extracting high-quality pectin, renowned as a vegetarian gelatin substitute. They can be used in nutrition and to make useful by-products that can be used in many different industries. Lime acids possess the capability to react with amines in fish, forming non-volatile ammonium salts. Additionally, these acids demonstrate the ability to hydrolyze resilient

collagen fibers. Notably, the primary mineral in lime juice is potassium (Liu et al., 2022).

As an important antioxidant source of lime, flavonoids are thought to have a stronger neuroprotective effect as they change the signals inside neurons that control their survival, death, and differentiation. Flavonoids are prevalent in plant tissues, primarily occurring in relatively high concentrations as sugar conjugates. The rutinose flavanones found in lemons and limes contribute to a neutral flavor, with neohesperidin being a distinctive component exclusive to limes. Eriocitrin is one of the glycoside flavonoids that has the best antioxidant activity. In lime, hesperidin is the main flavanone. It has been revealed that lime oil contains 15.64 mg/100 g of hesperidin (Liu et al., 2022). Oil from lemons and limes encompasses oxygen-containing heterocyclic compounds. Bioactive substances and phytochemicals, like hesperidin and eriocitrin, have been shown to improve oxidative stress and lipid profiles, which is especially helpful for people with metabolic syndrome. Also, coumarins and furocoumarins, especially bergamottin and 5-geranyloxy-7-methoxycoumarin parts, have strong anti-tumor and anti-cancer properties (Liu et al., 2022). This shows that lime components have a lot of different drug-like potential. The acidity of lime fruit depends on the carbohydrate concentration in the fruit (Balyan et al., 2024). This acid was found to maintain the quality properties of gel coats (Yousefi et al., 2024).

The peels of the lime types were all different in terms of phenolic content. However, it was more abundant in bioactive compounds, as indicated by their higher antioxidant capacity (Cioni et al., 2022). Mexican lemons showed a strong positive correlation between antioxidant capacity and phenolics (Mohammed et al., 2024).

The research identified myristic acid, 1,4-butanediol, *n*-hexadecanoic acid, 1,2-benzenedicarboxylic acid, diisooctyl ester, stigmasterol, and spathulenol by gas chromatography combined with mass spectrometry. *N*-hexane was used as the solvent and a Soxhlet extractor. Other lemons do not contain the terpenoids found in these extracted oils from lime seeds. However, the lime seed did not contain flavonoids, phenolics, or tannins. Lemon seeds and peel had greater antioxidant activity and proportion of tetradecanoic acid (Oluwatobi et al.,

2023). Furanocoumarins are not present when distilled essential oils are used. However, the expressed oils pose a modest hazard to phototoxicity (Lin et al., 2019).

2.2. Essential oils

Essential oils contain terpenoids or isoprenoids, which are many plants' most common and structurally varied natural compounds with various structural variations (Powder-George, 2024). Terpenoid groups such as monoterpenes, sesquiterpenes, and oxygenated terpene derivatives are most of the chemicals in essential oils. Additionally, oxygenated terpenes, which include alcohols, aldehydes, and ketones. Essential oil compounds are influenced by plant genetics and numerous environmental stress factors, as proven in research analyzing extraction methods (Julaeha et al., 2022b). However, limonene is a key component (Etta-Francis et al., 2022). Therefore, it's worth noting that *d*-limonene is highly preferred in the industry due to its numerous properties (Bellisai et al., 2021).

UV-A irradiation tests with bergamot, lemon, and lime oils have shown that furocoumarins have photo-protective properties for terpene stability. Keeping important molecules like *R*-(+)-limonene and γ -terpinene from breaking down too quickly stops the formation of unwanted oxidation products like limonene hydroperoxides and *p*-simene. This outcome holds considerable importance, given the pivotal role of odor in the essential oil, perfume, and cosmetics industries. Consequently, the natural presence of furocoumarins in *citrus* and other essential oils contributes to extended shelf life and enhanced stability in the final consumer products (Bitterling et al., 2022).

The beneficial impact of natural furocoumarins in cold-pressed citrus oils is particularly notable, owing to their photo-protective effects on essential oils. Furthermore, these furocoumarins can serve as effective photo-protective agents when added to highly sensitive essential oils. Acting as a protective shield, furocoumarins shield sensitive terpenes from direct exposure to UV radiation, effectively absorbing most radiation energy. Additionally, fluorescence deactivation is associated with a pronounced bathochromic shift to wavelengths exceeding 400 nm. Consequently, low-energy radiation lacks the potency to initiate direct terpene oxidation (Bitterling et al., 2022).

Furocoumarins also reach the triplet state through intersystem transition (ISC), which makes it easier for high-voltage UV radiation to be turned into lower-energy visible light. This mechanism significantly favors the stability of terpenes, further emphasizing the photo-protective role of furocoumarins in essential oils. The component γ -terpinene exhibited a relatively abundant presence in all three oils, whereas the degradation of *R*-(+)-limonene occurred at varying rates, with lime and lemon oils experiencing. Notably, *R*-(+)-limonene, a monocyclic monoterpene, is highly susceptible to dehydrogenation, rendering it particularly sensitive to degradation. The undesirable formation of *p*-simene from terpinene is associated with an unpleasant kerosene-like odor. Adding furocoumarins significantly mitigates the loss of γ -terpinene while simultaneously reducing the formation of the unpleasant compound *p*-simene. Concurrently, a notable formation of hydroperoxides was observed. Beyond preservation, furocoumarins' protective impact improved olfactory quality, possibly due to a bathochromic shift of radiated light towards fewer harmful, wider wavelengths. The fact that stable furocoumarins and unstable terpenes work together shows that photosensitized citrus compounds can be stored for longer (Bitterling et al., 2022).

The lime peel essential oil contains triterpenoid steroids (Julianti Wijayadi and Rusliati Rusli, 2020). In a study analyzing 36 constituents by gas chromatography combined with mass spectrometry, the five major components of *C. aurantifolia* oil were *d*-limonene, cyclohexene, 4-methylene-1-(1-methylethyl), citral, *cis*-citral, and α -farnesene (Julaeha et al., 2022a). In the study with 33 mg/mL, the lime solution was prepared as the median lethal concentration, the amount of essential oil was determined as 44.74%, and α -terpineol was determined by gas chromatography combined with mass spectrometry (De Paiva Silva et al., 2023). The study set the instrument conditions as a 45-400 mass load range and the mass spectrum time as 0.5 seconds. Relative retention indices were defined based on C₉-C₂₀, C₆-C₂₆ (Hassanein et al., 2023), and C₅-C₂₅ (Lin et al., 2019), n-alkane sequences. The monoterpene alcohol α -terpinol was the main component of lime (Da Silva et al., 2022).

Among the 18 components in lime peel oil, the highest amount of α -terpineol, non-polar *d*-limonene, β -pinene, and citral components affected

by geographical conditions were found. In the microencapsulation process, the oil yield was 51.46%, and the oil content was 58.66%, the essential oils found in lime (*C. aurantifolia*) peel included α -pinene (81%), β -pinene, β -phellandrene, (+)-4-carene, *d*-limonene (35.98%), *o*-cymene, β -ocimene, γ -terpinene, 2-carene (1.61%), β -myrcene, terpinen-4-ol, and β -myrcene (Sandra et al., 2020). The oil consisted of 39.23% *d*-limonene, 22.82% β -pinene, 5.63% citral, and 3.74% α -terpineol (Julaeha et al., 2021a). Key components include *d*-limonene (Mohammed et al., 2024), β -pinene, and terpineol (Da Silva et al., 2022).

Lime (*C. aurantifolia*) oil volatiles were found to be 98.3% monoterpenes. In the study, the monoterpene components limonene and citral are hydrophobic with a ring structure. The mixture in lemon oil was also significantly protective against *Staphylococcus aureus* (G+) (Allam et al., 2022). The lime essential oil contains 36% oxygen compounds. These compounds have strong antimicrobial activity compared to nitro compounds (Mohammed et al., 2024).

Citral, geraniol, linalool, and thymol are among the components found in Orange (*C. sinensis* L.O), Lemon (*C. limon* L.O), and Lime (*C. aurantifolia* L.O) fruit peel oils that show more phenolic and antiseptic properties. Additionally, the study found saponification values of 146 mg KOH/g, 158 mg KOH/g, and 123 mg KOH/g. The moisture content of pectin extracted from orange, lemon, and lime peels was 3.2%, 3.7%, and 4.4%. The pectin derived from orange peel and lemon peel was light brown, but that obtained from lime peel was dark brown. It is suggested that it can be supported as a supplement in animal feeding. Based on the research data, it is predicted that this study will positively impact future studies due to its low acid values, high oil yield rates, and excellent storage life (Etta-Francis et al., 2022).

Limes and lemons have different peel oil compositions (Jungen et al., 2023). The essential oil of lime also has a natural analgesic effect (Harahap et al., 2023). Higher photocatalytic activity was observed for the components with lime added (Hidayat et al., 2024).

D-limonene, phytol, α -tocopherol, and 5,7-dimethoxycoumarin have been identified in lime peels extracted with ethyl acetate, chloroform, and n-hexane solvents. Many of these are of biological

importance, such as stigmasterol, *d*-limonene, vitamin E, and α -tocopherol (Asmah et al., 2020).

The active chemicals discovered in the study on *C. aurantifolia* (Christm.) species included germacrene isomers (61.2%), pineene, linalool dimer, bornane, citral, anethole, anisole, safrol, and demitol. Lime peel oils, considered beneficial to human health, should be used in specific doses. However, the lime essential oil is thought to have mild hematotoxic, nephrotoxic, and hepatotoxic effects (Adokoh et al., 2019).

Compounds such as β -bisabolene, (*E*)-caryophyllene, geranyl acetate, trans- α -bergamotene, and α -humulene can decrease radionuclides (Salem et al., 2024). Lemon peel essential oil had an average repellency of 70.0 to 94.0 mg/cm². The absence of phytotoxicity suggests that these discarded waste essential oils might be used to safeguard the storage core (Visakh et al., 2022).

3. Essential oil extraction methods

Both conventional and unconventional methods are used in the extraction of essential oils. Conventional techniques include Hydro-distillation, Soxhlet Extraction, Cold Pressing, and Solvent Extraction. In contrast, non-conventional methods include supercritical fluid extraction, microwave-assisted hydro-distillation, solvent-free microwave extraction, microwave hydrodiffusion, and gravity. Hydro-distillation is the most commonly utilized method among these techniques (Figure 2) (Julaeha et al., 2022a).

Various physical properties, such as an increased refractive index due to longer chain components or oxygen-containing mechanisms, influence the composition of essential oils. These oils contribute to a higher medium density, impeding the refraction of incident light. The weight percentage of constituent components also affects physical properties specific gravity, and the acid number indicates the amount of free acids in a solution. In addition, most of the chemicals found in essential oils, such as β -pinene and *d*-limonene, are non-polar, which is anticipated to affect their solubility in alcohol (Sandra et al., 2020).

3.1. Distillation

It is housed in a distillation flask attached to a water-filled balloon with a round bottom. Pipes attach the condensation unit to the distillation

bottle. The essential oil is distilled using steam and filtered through the shells. After using an extraction condenser to extract the water phase, the diethyl ether phase is gathered, and the water is dried using a few drips of anhydrous magnesium salt. After that, the fat is measured and sent to a vapor extractor to remove any leftover diethyl ether (Etta-Francis et al., 2022).

A steam distillation study yielded 2.3% (w/w) volatile oil (Lin et al., 2019). The distillation method identified components such as citral, carveol, terpinen-4-ol, *p*-cymene, sabinene, and pinene at a higher rate than other *citrus* species (Dmitrieva et al., 2024). Sabinene, β -pinene, and limonene were the most prevalent volatile components in the essential oil derived from hydrodistilling Kaffir lime (*C. hystrix*). This kind of lemon's attractive aroma and high concentration of minerals and bioactive substances make it a valuable ingredient in nutritional supplements in capsule form (Lubinska-Szczygel et al., 2023). *C. aurantifolia* rip extract also has antioxidant activity and stabilizes membranes by gelling free radicals (Oyinloye et al., 2024). Specifically, the ratio of phenolic acids, the kind or quantity of flavonoids, and the absence of furanocoumarins are key requirements for the technique (Lee et al., 2022).

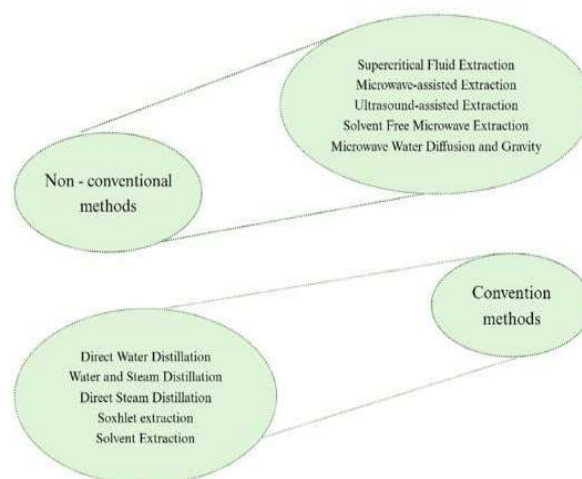


Figure 2. Extraction methods

3.2. Cold pressing

These methods are mainly employed for laboratory purposes, which involve pressing the flavado layer by hand to expose the lipid bubble. The fat can be gathered in saltwater and kept frozen. However, this method has not been widely used due to lesser yields than solvent, steam, or hydro-distillation

procedures (Etta-Francis et al., 2022). The industry faces a noteworthy challenge in employing the cold-pressing method due to the exceedingly thin peel of these limes. Consequently, a two-step process is adopted, wherein initial pressing yields an emulsion of juice and oil. Subsequently, direct steam distillation separates the lime oil from the juice (Liu et al., 2022).

3.3. Ultrasound-assisted extraction

In research utilizing *C. aurantium* (bitter orange) and lemon and orange tree leaves, analytes were extracted in methanol using an ultrasound-assisted extraction procedure. The study employed ultrasound-assisted extraction-capillary liquid chromatography for the first time to analyze limonene, linalool, farnesene, α -pinene, and myrcene components. The shorter analysis time provides an advantage over other approaches. Gas chromatography is commonly used to analyze terpenes in plants and plant-derived products. The terpene concentrations observable by gas chromatography-mass spectrometry are comparable or slightly lower. However, this is partially related to the preconcentration produced by solid-phase extraction or solid-phase microextraction techniques. The suggested capillary liquid chromatography approach has several benefits over gas chromatography-based diagnostics, including simplicity and speed. As a result, the approach may be used to analyze biological activity attributed to particular terpenes like those used in this study. It may also be used to compare the biological activity of various items by estimating the total quantity of certain terpenes. Although terpenes were the only substances evaluated in this work, it is worth noting that capillary liquid chromatography is a flexible technology that may be used to determine volatile and non-volatile plant elements simultaneously. The main constraint is the need for a capillary liquid chromatography system, which provides advantages in analytical performance and application. This approach positively influenced biological activity, raw material quality, storage conditions, and processes (Ponce-Rodriguez et al., 2021).

Lemon juice was centrifuged, and the solid residue was sonicated in 1 ml of fresh acetonitrile at 20°C for 5 min. The samples were passed through methanol cartridges. A serum extract was prepared by eluting the retained oxygenated heterocyclic

compounds with ethyl acetate. Ultrasonication (300 w, 20°C, 5 min) was used to redissolve the dried extract in methanol. At the end of the study, the oxygenated heterocyclic compounds 8-geranyloxypsoralen, byacangelicin, 5-geranoxy-7-methoxycoumarin, phellopterin, and methoxyflavones in lemon and lemon juice were characterized. These compounds have also been proven to possess medicinal properties (Li et al., 2021).

3.4. Supercritical fluid extraction

When essential oils dissolved in aqueous media undergo thermal-oxidative processes, inactive components are formed. One study focused on the antidiabetic properties of lime oil using treatment with polyethylene glycol and lauric acid via supercritical carbon dioxide at 120 psi and 45°C. Polymer-oil combinations produced co-precipitates, were then micronized using a 500 μ m nozzle. The addition of polyethylene glycol and lauric acid to lime oil in a gas-saturated solution made spherical microparticles that were approximately 2 μ m in size. The encapsulation of these particles resulted in a lower melting point and fusion heat than those enclosed with just polyethylene glycol. This has resulted in enhanced oil holding capacity and performance. The encapsulation procedure increased the average release time, reducing evaporation in physiological fluids. The ability of encapsulation to protect the anti-free radical and α -amylase inhibitory effects of essential oils shows that they can keep their medicinal properties (Akolade et al., 2020). Distillation, cold pressing, microwave-assisted extraction, and supercritical fluid are generally applied as extraction methods for essential oils (Razola-Diaz et al., 2021).

3.5. Microwave extraction

The microwave-extracted oil from the sample was cooled in the condenser. The condensate was routed via a separation funnel to separate the oil from the water. A vacuum pump produced the necessary suction pressure. Optimal circumstances were discovered with 797,844 W microwave power and 500 to 1000 W microwave power for approximately 30 minutes. Under these settings, the essential oil yield was 0.792 ± 0.03 %, and antibacterial activity was 18.25 ± 1.45 mm. In addition, delicious lemon peels were ultrasonically pretreated before microwave extraction. As a result, the essential oil yield rose from 0.84% to

1.06%. *D*-limonene, bergamol, β -pinene, linalool, α -pinene, 1,8-cineol, and α -terpineol components were identified in sweet lime peels in the highest ratio (Arafat et al., 2020).

4. Encapsulation

Microcapsules are formed through the attraction between oppositely charged molecules, creating an insoluble colloidal phase. The technique encapsulates the core with a polymer and reduces surface tension. During co-acervation, amide groups are formed when carboxyl groups in polysaccharides combine with amino groups in proteins. To ensure the presence of reactive ends toward specific functional groups, it is necessary to stimulate this need. A notable example is glutaraldehyde, which is often used to form covalent bonds between the carbonyl and amine groups in glutaraldehyde/gelatin. The formation of covalent bonds presence of cross-linking sites both affect microcapsule density. In particular, the mixing speed during the process is critical. Rapid particle movement promotes microcapsule bursting, resulting in a less homogeneous size distribution. This underscores the importance of carefully controlling mixing conditions to achieve the desired microcapsule characteristics (Figure 3) (Sandra et al., 2020).

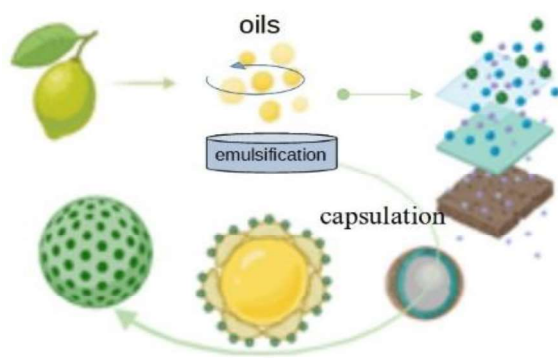


Figure 3. Encapsulation

Lime was identified using scanning electron microscopy, the content of *C. aurantifolia* essential oil within the microcapsules was determined using ultraviolet-visible spectrophotometry, and the oil content was calculated using regression equations developed from standard curves. Equations (1) and (2) are used to compute oil content and encapsulation efficiency, respectively (Julaeha et al., 2021a).

$$\left(\frac{\text{Actual coating oil amount}}{\text{weight of microcapsule}} \right) \times 100 \quad (1)$$

$$\left(\frac{\text{Actual coating oil amount}}{\text{amount of oil added}} \right) \times 100 \quad (2)$$

Lime seed essential oil capsules were round and supple, with a mean particle size of 1.554 μm . It is worth noting that 79% of the oil was discharged within 2 hours due to an interaction of mechanical and chemical forces. A 4% citric acid binding agent improved fixation, resulting in just 3% mass loss after 15 washes. The functional fabric coated with 4% binder had significant antibacterial action. This highlights the potential of utilizing essential oil microcapsules for developing antibacterial functional fabrics with enhanced durability through optimized binding methods (Julaeha et al., 2021b).

In the study demonstrating the suitability of *C. aurantifolia* shoot tips for encapsulating artificial seeds, the beaded shoot tips exhibited a maximum germination rate of 81.43%. Autoclaving at 121°C and 104 kPa, with pH correction to 5.8, proved effective for sterilization. The procedure for creating synthetic seed encapsulation from shoot tips was as follows: First, mix 200 mL of liquid seed sodium alginate solution with 30g of sucrose. This methodology is aimed at creating encapsulated synthetic seeds for further utilization in plant propagation and cultivation (Sharma and Roy, 2021).

A particle size analyzer can also be used to determine the size and distribution of microcapsules. All microcapsules can have well-distributed particle sizes and homogeneity based on mean/median ratios close to one. According to scanning electron microscopy, micrographs of oil microcapsules cross-linked with 15% CaCl_2 , mostly spherical and clustered microcapsules, were observed. Fourier may identify more efficient interactions to transform the infrared spectroscopy study of the microcapsules. Among all microcapsules, 15% of cross-linkers retained up to 73% of the essential oil core. The study observed a decrease in essential oil capsules stored for three weeks. This was thought to be due to physical and chemical changes in the coating, oil diffusion, and capsule disintegration. Moderate inhibition of *S. aureus*, *S. epidermidis*, *E. coli*, and *K. pneumoniae* bacteria was found. As a result, antibacterial oil microcapsules have a broad application in the cosmetic and textile industries (Pratiw et al., 2022).

The essential oils isolated from *C. aurantifolia* were packaged in gelatin alginate capsules. The microcapsules were immobilized on cotton fabric

by pad-drying with a citric acid binder. The microcapsules were tested for their antibacterial efficacy against gram-positive *S. aureus*. The findings revealed that the components of *C. aurantifolia* essential oils work together to enhance their effectiveness. The antibacterial activity of the microcapsules was comparable to that of the positive controls, ampicillin and limonene, indicating that the microcapsules can sustain antibacterial efficacy (Julaeha et al., 2022). In addition, as a result of a study with 58.66% oil content and 92.04% encapsulation efficiency, it is understood that more oil is absorbed in microcapsules of *C. aurantifolia* bark essential oils in 1:2.0 ratios of gelatin and sodium alginate coatings (Sandra et al., 2020).

Essential oils are fragrant, fatty organic compounds produced naturally in plants. Microcapsules play an important role in preserving and using essential oils' antibacterial and insecticidal properties in various sectors. Microcapsules help to preserve food during long-term storage. In medicine, the prolonged release of encapsulated essential oils has shown efficacy against multi-resistant bacteria, skin irritation, and cancer cells in animals. Furthermore, in the textile business, fabrics containing encapsulated essential oils have demonstrated remarkable repellency against mosquitoes and microorganisms and UV protection, even after several washing cycles (Nguyen et al., 2021).

The Food and Drug Administration classifies essential oils as medicines based on their intended use. As a result, the coating procedure is crucial in determining average size, stability, encapsulation efficiency, and release profile. Essential oils' nature, amount, and stereochemistry are significantly altered during extraction. Several factors must be addressed when establishing an acceptable delivery system for individual essential oils, including the manufacturing process (heat or organic solvent) (Cimino et al., 2021).

The effect of adding nanogel loaded with gelatin and lemon peel extract, composed of guar gum and gelatin, to a casein/basil seed chewing gum film revealed significant nanoparticle accumulation on the surface at higher percentages. Regardless of concentration, the films containing nanogel exhibited good antioxidant properties (Mei KR., 2021), which are considered positive for coating techniques. Coating processes conducted at various

concentration levels contribute to extending the final product's shelf life. Green technology views encapsulation as a valuable approach to enhancing food safety (Mortazavi Moghadam et al., 2023).

Polymeric nanoencapsulation is a widely adopted strategy for preserving essential oils, and among the various techniques for designing polymeric nanoparticles, nanoprecipitation has garnered significant attention. Putting essential oils inside polymeric nanoparticles makes them better at many important jobs, like killing microorganisms, stopping oxidation, fighting fungal infections, lowering inflammation, and getting rid of pests and insects. This approach, employing polymeric nanoparticles for delivering essential oils, represents a cutting-edge development in pharmaceutical technology. Additionally, scaling up nanoprecipitation for industrial applications is essential to implementing this technology on a larger scale (Lammari et al., 2020).

Copper oxide nanoparticles were synthesized in research utilizing *C. aurantifolia* leaf extract. The biosynthesized CuO nanoparticles had medium crystalline characteristics, with a crystal size of roughly 22 nm and a band gap of 3.48–3.51 eV. (Rafique et al., 2020).

The size of the nanocapsule oil obtained in the study was about 28 nm. A nano-emulsion is a heterogeneous mixture of a sparsely dispersed liquid in droplets in another liquid. These nano-emulsion droplets are measured between 20 and 500 nm. The surfactants Tween 80 and Span 20 were used for the encapsulation study in the dextrose agar medium (Allam et al., 2022).

Protect against toxin-induced biochemical changes with lime peel oil and nano-emulsion (Sabry et al., 2022). Essential oils' components are responsible for their pharmaceutical qualities. Some of the most researched qualities include antioxidant, anti-inflammatory, antibacterial, wound healing, and anxiolytic activity. Encapsulation helps to increase bioavailability, improve chemical stability, and reduce the volatility and toxicity of essential oils. Recent research has focused on various encapsulation strategies, such as micro- and nano-emulsions, liposomes, solid lipid nanoparticles, and nanostructured lipid carriers, to improve essential oil delivery and therapeutic potential (Cimino et al., 2021).

New food packaging trends include encapsulated bioactive essential oil components. This method improves food shelf life by utilizing essential oils' significant antibacterial and anti-pathogenic qualities (Shaaban and Farouk, 2022). The shelf life of the lime fruit in the coating is increased by the combination of pomegranate seed oil and gelatin (Mohammadi et al., 2024). A new composite of lime peel oil, whey protein, gum arabic, carboxymethyl cellulose, and gelatin was found to have favorable nanocapsulation (Hassane et al., 2023). Essential oil coating inhibited fungal spores on rubberwood (Owolab et al., 2021)

4.1. Encapsulation methods of lime oil

Research has revealed that limonene is extremely efficient against a wide spectrum of bacteria and fungi, often outperforming other terpenes, extracts, and essential oils. Its applications in food processing, storage, and packaging are significant. Encapsulation may be the best way to maintain its action and prevent oxidation. These include simple and sophisticated co-acervation, nano- or microencapsulation with various wall materials, including polysaccharides or proteins, molecular entrapment with cyclodextrins, spray drying, electrospinning, and nano-emulsion. Less prevalent methods include electrospraying and supercritical fluid technologies (Ibáñez et al., 2020).

4.1.1. Coacervation

One significant advantage of this approach is that the encapsulated limonene's form, size, and release rate may be adjusted by simply adjusting the concentration and ratio of chitosan and NaOH solution. Release profiles are typically created in two stages. Encapsulation via co-acervation, which provides a good barrier against the oxidation of sensitive components, looks to be an excellent method for encapsulating limonene flavor. The most critical criteria for creating co-acervation biopolymer complexes with maximum limonene encapsulation are viscosity and pH (Ibáñez et al., 2020).

Microcapsules containing *C. aurantifolia* essential oil, alginate/gelatin polymers, and different concentrations of CaCl_2 cross-linking agents were optimized for essential oil co-acervation utilizing Tween 80 emulsifying agents. The study used Avrami's kinetic equation ($k=1.60\pm3.68 \times 10^{-5}\text{s}^{-1}$) to simulate the microcapsule structure and release

mechanism, with an average particle size of $1.394 \mu\text{m}$. 43.56% encapsulation effect was observed at ambient temperature for three weeks, showing good thermal stability up to 100°C . Combining essential oil and alginate-gelatin shells containing varying concentrations of CaCl_2 cross-linking agent resulted in a microcapsule yield of 35-41%. The optimum number of microcapsules was obtained at 10-20% crosslinker (Pratiwi et al., 2022).

Essential oil capsules were obtained through complicated co-acervation with two biopolymers: alginate-gelatin, gum arabic-gelatin, and gum arabic-chitosan as shell, all under identical conditions. The alginate/gelatin combination yielded the most spherical and thinnest microcapsules, ranging in size from 5 to $33.1 \mu\text{m}$ without any aggregation. The gum Arabic/gelatin shell pair resulted in coarse and partially agglomerated microcapsules in the $7\text{-}10.1 \mu\text{m}$ range. Gum arabic/chitosan exfoliants are small clustered microcapsules ($2.1\text{-}5.1 \mu\text{m}$) dispersed on the surface. Because essential oils are often soluble in non-polar solvents, n-hexane was utilized to extract the released essential oils for measurement. The alginate/gelatin biopolymers produced the best essential oil microcapsules with well-dispersed particle size and homogeneity (Julaeha et al., 2022a).

The mixed conservation method study used separate 2% alginate and gelatin coatings. To achieve a $60\pm1^\circ\text{C}$ temperature, and agitated at 1250 rpm. Tween 80 (0.8g) and essential oils were combined in drops. The sodium alginate solution (40 mL) was then added gradually. The resultant liquid was agitated for 15 minutes before adding 2.5% acetic acid to achieve a pH of 3.75. The resultant microcapsules were then filtered via a Buchner funnel. The finished product was washed with demineralized water. Research has reported that microcapsules made with this technique can function as oil mimics (Sandra et al., 2020).

The peel oil of lime was microencapsulated utilizing the co-acervation procedure, which used gelatin and alginate as the wall material at 2% (w/v), Tween 80 as the emulsifier, and calcium chloride as the cross-linker. The diameters of these capsules ranged from 52 to $178 \mu\text{m}$. The increase in the amount of oil did not affect the diameter of the capsule particles, and the variation of the microcapsule yield between 26% and 31% was due

to the different weights of the essential oils. Particle size and morphology were also affected by processing at various mixing temperatures. Microcapsules generated at 35°C had a slightly lower particle diameter than those created at 40°C, but the shape was more uniform and spherical. Different mixing speeds resulted in microcapsules measuring 1.40-1.52 µm and yielding 23-30%. Increasing the speed beyond 600 rpm destroys the encapsulated oil. This decreases both oil content and yield. The mixing method controls both the microcapsule size and the size distribution (Julaeha et al., 2022a).

The viscosity of the emulsion is reduced, and the demulsification rate is increased by increasing the citric acid content. Citric acid has a higher emulsifying efficiency than other acids due to the high number of carboxyl groups. Suspended droplets coalesce, and the emulsion separates easily, with a balanced separation between the aqueous and emulsion phases. Each emulsification is optimized at 80°C. An inexpensive emulsifier is an effective method of remediating contaminated water (Moodley et al., 2022).

The microemulsions were then impromptu prepared by combining all the components and mixing in a vortex mixer. The research were formed using decyl glucoside and butylene glycol as surfactant and co-surfactant in a 2:1 weight ratio. The irritancy of these oils was shown to be reduced by microencapsulation (Prommaban and Chaiyana, 2022).

4.1.2. Spray drying

The spray drying method results in uniform morphologies due to the effects of wall composition, atomisation, drying parameters, uneven shrinkage in the first drying stages and the directed surface pressure of the viscous fluid (Compelo-Felix et al., 2017).

The essential oils of lime peel (*C. aurantifolia*) are complex metabolites used as preservatives. Physical methods such as spray drying, lyophilisation, supercritical fluidisation, and solvent evaporation are examples of physicochemical techniques: co-conservation, ionic gelation, and electrostatic film formation. Chemical approaches include interfacial polymerisation and molecular inclusion complexation (Da Silvet al., 2022). An encapsulation technique has proven effective in

protecting essential oils from environmental conditions, including oxidation and biological activity. In the industry, spray drying and emulsification are among the most preferred methods for encapsulation (Shaaban and Farouk et al., 2022).

4.1.3. Electrospinning technique

Increasing the polymer content via electrospinning will either raise the viscosity of the emulsion or precipitate the polymer, decreasing or even eliminating the probability of the encapsulated limonene spreading. Relative humidity was discovered to have an important impact on component release. This makes this approach appealing in active packaging, particularly for fresh foods, where the risk of microbial deterioration is greater in environments with high water activity. Emulsion electrospinning is utilized to create animal serum albumin-controlled delivery systems. One of the most significant benefits of employing supercritical fluid technology to encapsulate limonene is that it operates at low temperatures, allowing sensitive compounds to be encapsulated. The efficiency of encapsulating the limonene component was lower than that of conventional spray drying techniques. Limonene's preservation properties, health advantages, and pleasant taste and odor make it valuable in the cosmetics, food, and beverage sectors. It has proven antimicrobial activity in food products as well as antioxidant potential in preventing decay and extending shelf life. With its broad biological activities, limonene's non-toxicity and numerous modes of action make it an attractive natural alternative to synthetic pesticides and preservatives for a growing industry. Ambient conditions highly influence this compound (Ibáñez et al., 2020).

4.1.4. Freeze-drying (lyophilization)

Lyophilization is a preferred method for essential oil extraction because it protects compounds from high temperatures. Among various extraction methods, ultrasound-assisted extraction is considered the best, followed by microwave-assisted extraction and agitation extraction. Essential oils are sensitive to high temperatures, and these methods help preserve their quality. Cold-pressed lime oil, obtained by mechanical pressing of the peel, contains citral, high levels of sesquiterpenes, and aldehydes. Monoterpenes are found in lower amounts in the oil from the peel than in the water, and approximately 20% of non-

volatile chemicals are present (Liu et al., 2022). With spray cooling, a thin coating film is formed around solid, liquid and gas particles or live organisms (Abdul-Al et al., 2022).

Contrarily, distillation does not yield essential oil that contains non-volatile substances. People are now more interested in the oxygen heterocyclic compounds found in lime oil. To check their quality, high-performance liquid chromatography analysis has replaced the older total UV absorption

methods. Different processing methods can influence the composition of these non-volatile compounds. It's noted that bergamottin, a compound found in lime oil, possesses natural anti-cancer properties. Therefore, caution is advised during extraction to avoid affecting its beneficial properties and overall metabolism (Liu et al., 2022). The data from the literature studies and the conditions of the research with lime peel oil are summarized in Table 2.

Table 2: *C. aurantifolia* application methods and their effects

Interfacial solvent/Wall material	Encapsulated material shape and size	Application method	Key findings	References
Distilled water: Copper sulfate pentahydrate	Irregular and spherical-like nanoparticles (22 nm)	Biological synthesis	Activity against <i>S. aureus</i> and <i>E. coli</i>	(Rafique et al., 2020)
<i>n</i> -Hexane- acetic acid; tween-80 /gelatin and sodium alginate	Microcapsules with various shapes	Complex co-acervation	Increasing the coating ratio increased encapsulation efficiency and yield	(Sandra et al., 2020)
Hexane, dichloromethane, dimethylsulphoxide; Polyethylene glycol and lauric acid	Rough spherical microparticles (~ 2 µm)	Gas-saturated solution technology	The overall average lime oil release time was increased. The encapsulation yield was raised.	(Akolade et al., 2020)
Tween 80, CaCl ₂ , acetic acid; alginate–gelatin	Spherical and agglomerated shape (1.394 µm)	Co-acervation	Gram-positive and Gram-negative antibacterial activity, effectively coacervated with 15% CaCl ₂ cross-linker	(Pratiwi et al., 2022)
<i>n</i> -Hexane, citric acid, Tween 80; glutaraldehyde, alginate-gelatin-gum Arabic-chitosan, sodium alginate	Spherical capsules (1.802±0.082/1.554 µm)	Pad-dry-cure method using various concentrations, Complex Co-acervation	Microcapsules covered with biodegradable polymers Moderate antimicrobial activity was detected. Superior bactericidal activity	(Julaeha et al., 2022)
<i>n</i> -Hexane, Tween 80, acetic acid; calcium chloride, gelatine, alginate,	Homogeneous and rounder morphology (1.40-1.52µm)	Complex Co-acervation	Microcapsules were optimally characterized and manufactured successfully.	(Julaeha et al., 2021)
Sodium periodate, ethylene glycol, acetone; casein, gelatin, guar gum, dialdehyde-guar gum	Smooth nanoparticle	Nanocapsulation; inverse miniemulsion synthesis	A novel nano gel was fabricated by inverse miniemulsion synthesis with an inhibitory effect against microorganisms.	(Mortazavi Moghadam et al., 2023)
Tween80, deionized water; oil phase (oil and corn oil)	Spherical shape (20-60 nm)	Nano-emulsion; spontaneous emulsification method.	Gram-positive microbes were more susceptible and could be used as functional ingredients.	(Mei K.R., 2021)
Glacial acetic acid, tween80, sodiumtripolyphosphate; chitosan	Homogeneously distributed (271.7 nm)	Nanocapsulation; ionic gelation method; freeze drying method	Prevents acne vulgaris and treats acne effectively and efficiently	(Julianti et al., 2020)

5. Conclusion

These fruits are cultivated commercially across diverse countries, including India, the United States, Spain, Italy, Argentina, Egypt, and Iran. In contrast, due to their sensitivity to cold climates, limes are exclusively grown in tropical regions, with Mexico and Brazil standing out as primary

producers. Due to their versatility and consistent demand, lemons contribute significantly to food safety while offering numerous health benefits. Recent advancements in extraction techniques aim to improve the quality yield of essential oils while simultaneously reducing energy consumption,

reflecting a progressive evolution in lime processing methodologies.

Lime's bioactive components benefit human health. However, these components also have active properties that ensure the safety and reliability of food products. Recently, oil extraction trials using green techniques have improved the quality of these sensitive-structure oils. Contemporary approaches use coating methods such as coacervation, spray drying, freeze drying, ve electrospinning to preserve the qualitative and quantitative attributes of essential oils extracted

from limes. Consequently, the composition of these oils and the selection of preparation methodologies stand as pivotal determinants in yielding a final product tailored for specific pharmaceutical applications.

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